BERTRAMIA BUFONIS, A NEW SPOROZOAN PARASITE OF BUFO LENTIGINOSUS.

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In a paper dealing with the structure and development of "Bidder's organ," a rounded body found at the anterior end of each testis in various species of *Bufo*, Knappe[1] states that occasionally spermatozoa are found in the cells of this organ which is undoubtedly a rudimentary ovary; he adds, furthermore, that these spermatozoa have probably been formed from small follicle cells which have entered the cytoplasm of the undeveloped ova.

In the course of investigations which I have been making this past winter upon the development of the germ-cells and the structure of Bidder's organ in the common American toad, *Bufo lentiginosus*, I have found one individual in which the cells of Bidder's organ contain bodies, unquestionably parasites, which are very similar to those figured by Knappe as spermatozoa. It seems probable, therefore, that in the material studied by Knappe the "spermatozoa" are stages in the life cycle of some species of Sporozoa; for in the light of our present knowledge regarding the origin and development of germ-cells it is inconceivable that functional spermatozoa could be formed in or from the cytoplasm of rudimentary ova that are destined to undergo degeneration. Whether the species of parasite found in the American toad is the same as that infecting the European form I have not been able to determine, as details of structure cannot be made out from the figures given by Knappe.

The individual infected by the parasite was a young male which was killed on July 16, 1905, at Owego, N. Y. Nothing unusual or abnormal about the toad attracted my attention at the time that the animal was killed; and, as material was being collected for a study of the genital organs, only the testes, Bidder's organ, and a portion of the kidneys were preserved. The material was fixed in Flemming's solution and stained with iron-hæmatoxylin followed by orange G.

As so few of the organs of the body were preserved, it is impossible to state the extent of the infection. The parasite is found only in the ova of Bidder's organ, and not in the testes or in the renal tubules.

[1] Knappe, E., Das Bidder'sche Organ, *Morph. Jahrb.*, Bd. XI, 1886.

Judging from the stages that I have been able to find, the parasite is undoubtedly a Sporozoan belonging to the order Haplosporidia. It does not fit in very well with the present scheme of classification of the Haplosporidia; but until its complete life history is known, I do not think it advisable to create for it a new genus. I shall, therefore, place it temporarily in the genus *Bertramia* (Caullery and Mesnil, 1897). As the parasite is apparently a new species, it may be known as *Bertramia bufonis*.

The youngest stage of the parasite that I have been able to find is a small, round or oval body with a diameter of about two microns (plate XXII, fig. 1, *a*). The cytoplasm is uniformly granular and the nucleus, which occupies the centre of the cell, is round or somewhat irregular in outline. The nucleus is composed, apparently, of a dense mass of chromatin, as it always appears homogeneous and stains intensely black with the iron-hæmatoxylin.

After the young parasite enters one of the ova of Bidder's organ it divides repeatedly, forming a number of cells similar to that shown in fig. 1, *a*. Cell division undoubtedly takes place by means of karyokinesis, as I have found several cases like that of fig. 1, *b*, in which faint traces of a spindle can be seen and also irregular masses of chromatin collected at the spindle poles. After the division of the chromatin, the daughter-nuclei assume a rounded shape (fig. 1, *c*), and subsequently division of the cytoplasm takes place. (fig. 1, *d*). Sometimes, as shown in fig. 1, *e* and *f*, one or both of the cells will have begun a second division before the first division is entirely completed. All of the stages shown in fig. 1, *a-f*, may be found in a single section of one of the large ova of Bidder's organ.

The period of multiplication is followed by a growth period in which each of the cells increases enormously in size and the nucleus divides a number of times without any corresponding division of the cytoplasm (figs. 2–5). The nuclear divisions at this stage of development are evidently also mitotic (fig. 3), although on account of the small size of the spindle and of the chromosomes it is quite impossible to make out any details of the process.

At a comparatively early stage in its development the trophozoite becomes surrounded by a thin membrane (fig. 4), which later forms a cyst wall enclosing the spores (fig. 7). The full-grown trophozoite has a diameter of 9–11 microns, and contains a large number of deeply staining nuclei which are usually irregular in outline (fig. 5). The multinucleated body soon segments into a mass of spores (fig. 6), which become round or oval as soon as separation is effected (fig. 7).

The mature cysts are about 15 microns in diameter and they are invariably rounded, unless, as occasionally happens, they are distorted by contact with adjacent cysts. Each cyst contains at least 30 spores. As the spores are closely crowded together, I have not been able to make out the exact number in any one cyst or to determine whether the number is constant for all of the cysts. The young spore has granular cytoplasm and a single, deeply staining nucleus (fig. 7, *a*). It resembles somewhat the stage of the parasite shown in fig. 1, *a*; but as it is considerably smaller and is always enclosed in a cyst, the two stages are easily distinguished. In their later history the majority of the spores follow one of two clearly marked modes of development, which, for convenience in description, will be designated as Type I and Type II.

The first change that takes place in the spores that develop according to Type I is the appearance of a clear area around the nucleus which occupies the centre of the spores (fig. 7, *b*). Owing to the small size of the spores it is impossible to determine whether the nucleus suddenly becomes vesicular at this time or whether the clear area is a fluid vacuole. Judging from the changes that take place in later stages of development, I am inclined to the opinion that the character of the nucleus does not change at this time and that the clear area around the nucleus is a vacuole. Soon after the formation of the vacuole the nucleus divides (fig. 7, *c*) and, as the spore elongates, the two nuclei separate and move to opposite ends of the vacuole which increases considerably in size (figs. 8, 9); subsequently the vacuole itself divides and each part comes to surround one of the nuclei (fig. 10). At the next stage the vacuoles, with the nuclei, are found at the ends of the spore which has now attained its final shape (fig. 11). The nuclei appear as large and stain as deeply at the stage of fig. 11 as they do in the young parasite; but in a slightly older spore they are considerably smaller and stain much less intensely (fig. 12). At a later period all traces of the nuclei are lost and the vacuoles appear perfectly clear and transparent (figs. 13, 14).

During all stages in the development of the parasite up to that of fig. 13, the cytoplasm appears granular and stains very faintly. When the nuclei disappear at the stage of fig. 13, the cytoplasm in contact with the vacuoles shows a strong affinity for the iron-hæmatoxylin, although the rest of the spore stains as faintly as before. In a slightly older stage the central portion of the cytoplasm stains as intensely as the end regions, and the entire spore, excepting the vacuoles, appears uniformly black (fig. 14). Somewhat later the vacuoles begin to

decrease in size (fig. 15) and eventually they too disappear. The mature spore (fig. 16) is about 3 microns long and 1.5 microns wide; the ends are oval and a slight constriction is usually found in the middle region. In a single section of a cell of Bidder's organ one may find as many as 15 cysts, containing spores in practically all stages of development, from that shown in fig. 7, *a*, to that of the mature spore shown in fig. 16. As cysts containing spores that stain very faintly may lie adjacent to cysts in which the spores are all stained black, it is evident that the great affinity of the cytoplasm for the iron-hæmatoxylin is not due to an overstaining of the material, but to some change taking place in the substance of the spore itself.

As the more intense staining of the cytoplasm of the spore is invariably coincident with the disappearance of the nuclei from the vacuoles, it would seem as if the two phenomena must be related in some way; and it is possible that, after the stage of fig. 11, the chromatin substance gradually becomes distributed throughout the cytoplasm and brings about a deeper staining of the spore contents. Although I have found a large number of spores in which the nuclei are of various sizes and stain with different degrees of intensity at the outer border of the vacuoles, I cannot be certain that the nuclei break down at this place; for I have found several spores like those shown in figs. 24 and 25, in which two nuclei lie in the cytoplasm after the vacuoles have moved to the ends of the spore. I have not succeeded in finding any stages that would seem to connect fig. 11 with fig. 24, yet it is possible that soon after the stage of fig. 11 the nuclei pass quickly from the vacuoles into the cytoplasm which at once stains more intensely. From the evidence at hand, I am inclined to believe that the spores shown in figs. 24 and 25 are abnormal and that the nuclei gradually disintegrate at the outer border of the vacuoles.

In the second type of development, which is not as common as the type just described, the nucleus moves to one end of the spore and takes a position as shown in fig. 17. A vacuole then forms around the nucleus as in Type I (fig. 18), and subsequently the nucleus divides (fig. 19). Later the two nuclei, which stain as intensely as in the earlier stages, move to opposite ends (figs. 21, 22) or, in some few cases, to opposite sides (fig. 20) of the vacuole. The vacuole increases in size as the spore elongates; but, as far as I have been able to determine, it does not divide into two parts, as does the vacuole in the spores that follow the first type of development. After the disappearance of the nuclei in the stage succeeding that of fig. 22 the vacuole gradually becomes smaller (fig. 23), and the entire spore stains black and appears as in fig. 16.

Deviations from these two types of development are not uncommon, some of the more striking variations being shown in figs. 24–31. The spore from which fig. 24 was drawn seems to be a variation of Type I, in which the nuclei have moved into the cytoplasm instead of towards the outer border of the vacuoles. It is possible, as I have already suggested, that fig. 24 shows a normal stage in the development of the spore, and that it belongs between the stage of fig. 12 and that of fig. 13. In the spore shown in fig. 25 it is evident that, when the vacuole divided, both nuclei remained in one of the two vacuoles that were formed (as is shown in fig. 28), and that they passed into the cytoplasm after the vacuole had taken its position at the end of the spore. As indicated in fig. 25, the cytoplasm surrounding the nuclei stains considerably darker than that in the rest of the spore; and although the nuclei are clearly seen at this time, they are totally obscured in the following stage when the region in which they lie stains black (fig. 26). A spore in which one nucleus only has left its vacuole is shown in fig. 27; while variations in the number, size, and situation of the vacuoles are shown in figs. 28–31.

I can offer no explanation as to the way in which the cells of Bidder's organ become infected with the parasite. There is no direct means of communication between this organ and the testis, and therefore it does not seem possible that infection was through the cloaca and the kidneys, hence by way of the testis to Bidder's organ. As Bidder's organ contains a large number of capillaries, it seems most probable that infection took place through the blood. Although I have carefully examined sections of the blood vessels containing large numbers of corpuscles, I have found no traces of the parasite either in the corpuscles or in the plasma. In one instance three mature spores were found in the plasma of a capillary in Bidder's organ; but it seems probable that these spores were derived from one of the many cysts in the organ containing ripe spores. As all of the large ova in Bidder's organ eventually degenerate, usually through the rupture of the outer wall and the penetration of a capillary into the interior, it is evident that the spores finally get into the circulation. How they pass to the exterior and whether they subsequently undergo a further development in another host remains to be determined.

During recent years much interest has been taken in the life history of the Sporozoa which seem to infect many of the invertebrates as well as all classes of the vertebrates. Interest in this group has naturally centred in the parasites infecting the mammals; and the amphibians as a class have not been systematically examined, either in Europe or in

America, for Sporozoan parasites. In the list of Sporozoa and their hosts recently compiled by Minchin,[2] *Bufo lentiginosus* is given as the host of one known species, *Leptotheca* (*Chloromyxum*) *ohlmacheri*, a Myxosporidian which was first described in 1893 by Ohlmacher,[3] who found it in the renal tubules. In but two other instances have Sporozoan parasites been found in any species of *Bufo*, and comparatively few cases of infection have been recorded for the different species of *Rana*. As presumably the amphibians are as subject to parasitic infection as are the other vertebrates, they would seem to afford a profitable field of investigation for the discovery of new Sporozoan forms.

Explanation of Plate XXII.

All figures were drawn with the aid of a camera lucida under a Zeiss apoc. obj. 1.5 mm. Oc. 8.

Fig. 1.—The young parasite and its division stages.
Fig. 2.—Trophozoite at the beginning of the growth period.
Fig. 3.—Mitotic division of the nuclei of the young trophozoite.
Fig. 4.—Formation of the cyst wall around the trophozoite.
Fig. 5.—Mature trophozoite before its division into spores.
Fig. 6.—Division of the trophozoite into spores.
Fig. 7.—Cyst containing young spores.
Figs. 8–16.—Stages in the development of spores according to Type I.
Figs. 17–23.—Stages in the development of spores according to Type II.
Figs. 24–31.—Spores showing apparent deviations from the usual modes of development.

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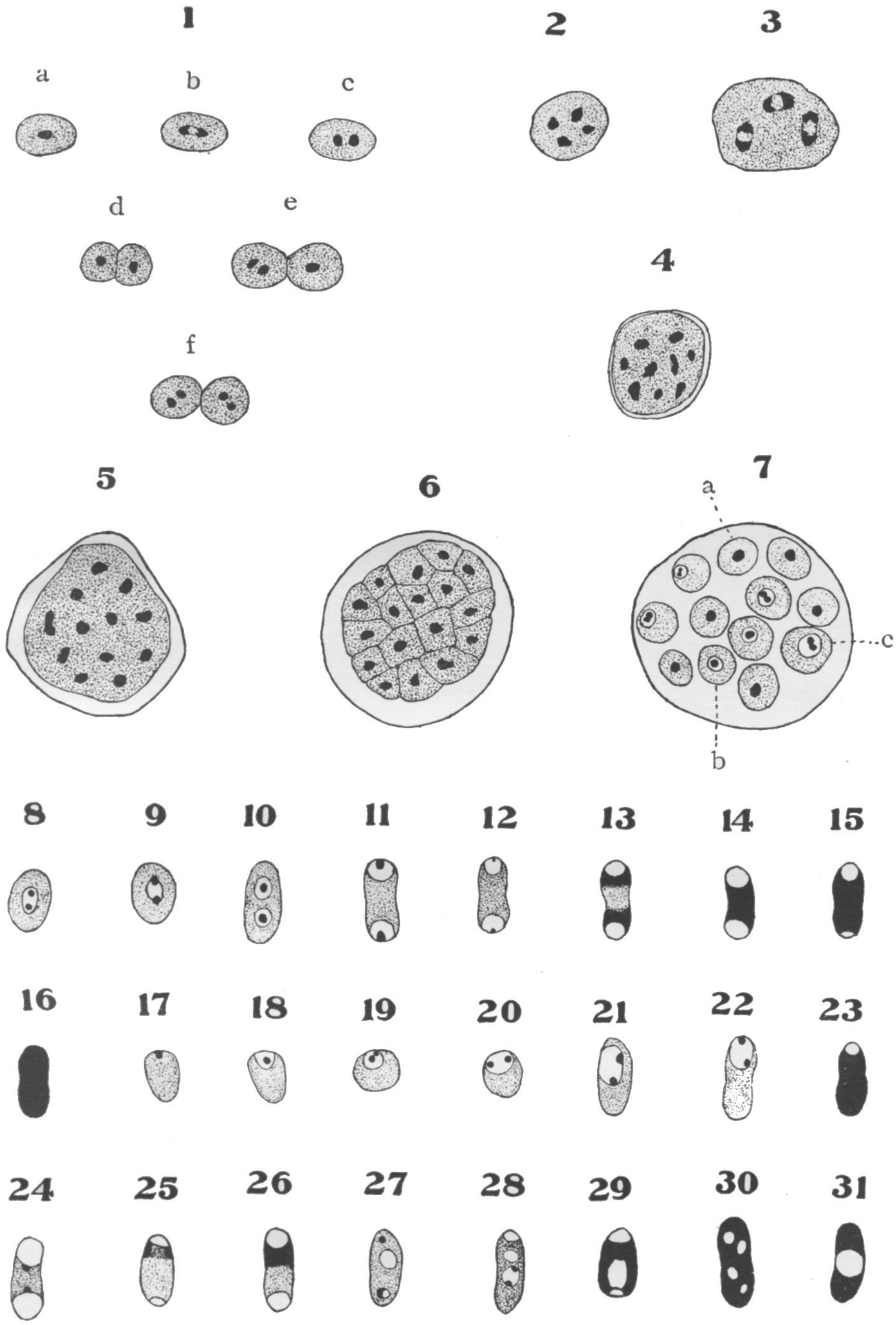
[2] Minchin, E. A., *Sporozoa: A Treatise on Zoology*, edited by E. Ray Lancaster, Pt. I, 1903.

[3] Ohlmacher, A. P., Myxosporidia in the Common Toad, with Preliminary Observations on the Chromophilis Substance in the Spore, *Journ. Amer. Med. Assoc.*, Vol. XX, 1893.



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